

OPTIMIZATION OF SUPERCRITICAL FLUID EXTRACTION PROCESS FOR MORINGA (PKM-1) SEED KERNEL OIL

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ABSTRACT

Oil was extracted from moringa (PKM-1) seed kernel by using supercritical fluid (SC-CO₂) extraction process. Supercritical fluid pressures of 100, 150 and 200 bar and temperatures of 40, 50 and 60 °C were considered for optimization of the extraction process. The extraction yield and extraction efficiency varied in the range of 31.87 to 37.76 g/100 g and 83.32 to 98.43%, respectively. Maximum extraction yield of 37.76 g/100g and extraction efficiency of 98.43% were obtained at supercritical fluid pressure of 200 bar and temperature of 40 °C. The desirability at optimum operating condition was found to be 0.98. Supercritical fluid extraction of moringa seed kernel oil was found to be an efficient and more convenient method as it is non-toxic and easy to separate the solvent from extracted oil by just depressurization. The production cost of oil from moringa (PKM-1) seed kernel using supercritical fluid extraction equipment was estimated and benefit cost ratio was found to be 1.83:1.

KEYWORDS: Supercritical Fluid, Carbon Dioxide, Extraction, Yield, Efficiency, Optimization

INTRODUCTION

Moringa (*Moringa oleifera* Lam) belongs to the family “*Moringaceae*” with genus “*Moringa* Adans” and species “*M. oleifera* Lam”. It is well known to the ancient world, but only recently it has been rediscovered as a multipurpose tree with a tremendous variety of potential uses. India is the largest producer of moringa, with an annual production of 1.10 to 1.30 million tonnes of tender fruits from an area of 380 km². Among the states, Andhra Pradesh leads in both area and production (156.65 km²) followed by Karnataka (102.8 km²) and Tamil Nadu (74.08 km²) (Lalas & Tsaknis, 2002).

Furthermore, *M. oleifera* seed has been found to be a potential new source of oil especially with the advent of the need for oleo-chemicals and oils/ fats derived fuels (Biodiesel) all over the world (Anwar & Rashid, 2007). However, the plant has been identified as one of the under explored plant.

There are a number of conventional extraction methods for essential oil extraction from plant materials. Some methods have been used for many years such as Soxhlet Extraction (SE), Heat Reflux Extraction (HRE) and Steam Distillation (SD). The main disadvantage of conventional extraction methods include long extraction time, usage of a large amount of solvent and the possibility of thermal decomposition of the target compounds (Qun, 2011).

Solvent extraction is being practiced for extraction of oil from moringa seed kernels. However, this method has the major disadvantage of solvent residue in the extracts. Recently, supercritical fluid extraction has gained increasing attention over the traditional techniques in the recovery of edible and essential oils. In the field of natural products, the new

technique of supercritical fluid extraction (SFE) utilises smaller amount of organic solvents. Supercritical carbon dioxide is an alternative that does not have any of the negative effects related to traditional organic solvents, at optimal conditions (Casas *et al.*, 2009).

To date, any research article on supercritical fluid extraction of PKM-1, a world's most successful high productivity variety of *Moringa oleifera* seed kernel oil has not been reported. Keeping in view of these facts, the investigation on "optimization of supercritical fluid extraction process for moringa (PKM-1) seed kernel oil" was undertaken in the Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur, Karnataka (India).

MATERIALS AND METHODS

Raw Materials

Clean and dried seeds of moringa (PKM-1) were procured from M/s. Bharath seeds, Raichur (Karnataka). The shells were removed manually and the kernels were ground in a laboratory hammer mill to obtain fine powder (Nguyen *et al.*, 2011). The solvents, chemicals and reagents (analytical grade) used throughout the experiment were procured from M/s. Sigma Aldrich Chemicals, Bangalore (Karnataka).

Soxhlet Extraction of Moringa Seed Kernel Oil

Moringa seed kernel oil extraction was carried out by soxhlet extraction method using SOCS- PLUS apparatus (Make: Pelican Equipments; Model: SCS-08) with hexane as solvent. Accurately, 50 g of the moringa seed kernel powder was taken into the thimble and placed it in the sample compartment of the extractor. Sample compartment was attached to a 500 ml round bottom flask containing 300-350 ml hexane. SOCS- PLUS set-up was assembled and heated in a mantle. The SOCS- PLUS apparatus was run at 85 °C for 90 min. Hexane in the oil extract was distilled out by using a rotary flash vacuum evaporator (Superfit, Rotavap; PBU-6D) (Malapit, 2010).

Supercritical Fluid (SC-CO₂) Extraction of Moringa Seed Kernel Oil

The supercritical carbon dioxide extraction system (Thar; SFE 500 system) was used for extraction of moringa (PKM-1) seed kernel oil. Deionized water (at 5 °C) was used for cooling different zones in the SC-CO₂ extraction system. The independent variables selected for the study were supercritical fluid (SC-CO₂) pressures of 100, 150 and 200 bar and temperatures of 40, 50 and 60 °C at constant dynamic extraction time of 90 min (Liza *et al.*, 2010).

Fifty grams of moringa seed kernel powder was placed into the extractor vessel. The flow rates of supercritical CO₂ and co-solvent (ethanol) were maintained at 20 and 2 g/min, respectively (Pradhan *et al.*, 2010). Static extraction process was performed for 30 min (Palafox *et al.*, 2012). After attaining desired pressure and temperature dynamic extraction time (90 min) was started by opening the exit valve of the SC-CO₂ extraction system. The static extraction time allowed the sample to soak in the CO₂ and co-solvent in order to equilibrate the mixture at desired pressure and temperature. During the dynamic extraction time, CO₂ carrying the crude extract flowed out of the extraction vessel and then into a collection vessel, where the CO₂ was separated through the vent connected to the fume hood.

Extraction Yield

The moringa (PKM-1) seed kernel oil from the SC-CO₂ extraction was collected and the residual content of co-solvent was removed by using a rotary flash vacuum evaporator (Superfit, Rotavap; PBU-6D) under vacuum at 40 °C. The

oil was then placed in the oven at 40 °C for 30 min for further removal of solvent traces. The extraction yield was computed by using the following equation (Orhevba *et al.*, 2013).

$$\text{Extraction yield (g/100g)} = \frac{M_{\text{extract}}}{m_{\text{feed}}} \times 100 \quad \dots \quad (1)$$

Where,

M_{extract} = Mass crude extract, g

m_{feed} = Feed mass, g

Extraction Efficiency

The extraction efficiency was calculated as per the method described by the Olawale, (2012). It is the ratio of the quantity of oil extracted during the process to the actual amount of oil present in 100 g of moringa seed kernel.

$$\text{Extraction efficiency (\%)} = \frac{\text{Oil extracted, g/100 g of sample}}{\text{Actual oil present, g/100 g of sample}} \times 100 \quad (2)$$

Optimization of Process Parameters

The numerical optimization technique of the Design-Expert software was used for the simultaneous optimization of the multiple responses. The desired goals for each variable and response were chosen. The desirability values of the minimum and maximum were configured as 0 and 1, respectively. All of the independent variables were kept within the range, while the responses were either maximized or minimized. Numerical optimization was applied for supercritical fluid extraction of moringa (PKM-1) seed kernel oil on the basis of extraction yield and extraction efficiency.

The quadratic response surface analysis was based on multiple linear regressions taking into account of linear, quadratic and interaction effects according to the equation given below;

$$Y = b_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} x_i^2 \quad \dots \quad (3)$$

Where Y is the response value predicted by the model; b_0 is offset value, a_i , a_{ij} and a_{ii} are main (linear), interaction and quadratic coefficients, respectively. The adequacy of the models was determined using model analysis; lack-of fit test and coefficient of determination (R^2) analysis (Mirhosseini *et al.*, 2009).

Verification of Predicted and Actual Responses

To verify the predicted and actual responses of SC-CO₂ extracted moringa (PKM-1) seed kernel oil, the mean relative per cent deviation modulus was calculated by using the following relationship (Krishnaiah *et al.*, 2012).

$$\text{Per cent relative deviation modulus} = \frac{100}{N} \sum_{i=1}^N \frac{|e_i - p_i|}{p_i} \quad \dots \quad (4)$$

Where,

N = Total number of observations

e_i = Experimental value

p_i = Predicted value

Statistical Design

The experiments were conducted with factorial design (3^2), which referred to two independent variables and three levels selected for each independent variable. Experimental data were subjected to analyses of variance (ANOVA) and multiple comparison tests were performed using a least significant difference (LSD), suitable for factorial design. The analyses were performed using the software, Design Expert Version 7.7.0 trial version (State-Ease, Minneapolis, MN).

Cost of Production of Moringa Seed Kernel Oil Using SC-CO₂ Extraction Process

The cost of production of moringa seed kernel oil using supercritical fluid extraction equipment was estimated by considering the fixed and variable costs as well as other related costs. The standard procedure in accounting and cost calculation given by Ababa *et al.*, (2004) was followed.

RESULTS AND DISCUSSIONS

Extraction Yield and Extraction Efficiency

The extraction yield and extraction efficiency of oil obtained from moringa (PKM-1) seed kernel powder at different SC-CO₂ temperature and pressure combinations are presented in the Table 1. The extraction yield and extraction efficiency varied in the range of 31.87 to 37.76 g/100 g and 83.32 to 98.43%, respectively. The interaction effect between different treatment combinations are significant ($p < 0.01$) at one per cent level.

Table 2: Effect of SC-CO₂ Temperature and Pressure on Extraction Yield and Extraction Efficiency for Moringa (PKM-1) Seed Kernel Oil

	Temperature (°C)	Pressure (bar)	Extraction Yield (g/100g)	Extraction Efficiency (%)
T ₀	-	-	29.12	76.29
T1	40	100	35.57 ^a	92.99 ^a
T2	40	150	36.86 ^a	96.36 ^a
T3	40	200	37.76 ^a	98.43 ^a
T4	50	100	32.14 ^a	84.02 ^a
T5	50	150	34.04 ^a	88.99 ^a
T6	50	200	35.26 ^a	89.56 ^a
T7	60	100	31.87 ^a	83.32 ^a
T8	60	150	32.38 ^a	84.65 ^a
T9	60	200	34.23 ^a	89.49 ^a
		Mean	34.52	89.88
		CD @ 1%	1.02	0.73
		CV	1.28	0.35

T₀ = Control - Soxhlet extraction carried out at 85 °C for 90 minutes

a= Significant at p<0.01

From the Table 1, it can be noticed that, as the supercritical fluid (CO₂) pressure increased from 100 to 200 bar, the extraction yield and efficiency increased. This might be due to the fact that the increase in pressure increased the density of the CO₂ thereby increasing the solvent strength and solubility of the oil in CO₂ (AOAC, 1990). Results are in agreement with the earlier findings of Nguyen *et al.* (2011) who reported optimal oil yield and extraction efficiency of 37.84 g/100 g and 90.90% at a pressure of 28.97 MPa and temperature of 44.30 °C for *Moringa oleifera* seed kernels. The increase in pressure might have also accelerated the mass transfer of oil and enhanced the extraction yield. Similar findings were reported by Zhao & Zhang (2013), who reported oil yield of 37.12% for *Moringa oleifera* seeds.

Increasing the temperature of SC-CO₂ reduced the solvent density and thus its salvation power at constant pressure (Couto *et al.*, 2009). It can be observed that, the extraction yield and extraction efficiency decreased with the rise of temperature at low pressures, due to the reduced density of CO₂ with increased temperature. However, in the present experiment at lower pressures 200 bar, the extraction yield and extraction efficiency decreased with the rise of temperature.

From the table, it is also seen that the moringa (PKM-1) seed kernel oil yield from solvent extraction (control) found to be lower (29.12 g/100g) with extraction efficiency of 76.29% compared to SC-CO₂ extraction. This might be due to lower solvation of oil by the solvent and higher temperature applied in soxhlet extraction compared to SC-CO₂ extraction. Higher temperature might have led to the thermal degradation of fatty acids, especially unsaturated fatty acids (Uddin & Chun, 2010).

Optimization of Process Parameters for SC-CO₂ Extracted Moringa (PKM-1) Seed Kernel Oil

The optimization work was designed for SC-CO₂ extracted moringa PKM-1 seed kernel oil using factorial design method in Design-Expert 7.7.0 software. The values of all the responses at operating conditions were converted to a desirability function. The desirability values of the minimum and maximum were configured as 0 and 1, respectively. The maximum desirability function obtained was taken as the optimum operating condition Krishnaiah *et al.* (2012). Optimization of the two process variables namely; temperature and pressure were performed using the general factorial method in Design Expert.

The optimized condition obtained was 40 °C temperature and 200 bar pressure. In the present investigation, the lack of fit was found to be significant for all responses. The responses were predicted by Design-Expert 7.7.0 software for the optimum process condition with desirability of 0.98. The desirability function of optimum operating parameters of SC-CO₂ extracted moringa (PKM-1) seed kernel oil at different treatment combinations are depicted in Figure. 1. From the figure, it can be noticed that, desirability of extraction of moringa (PKM-1) seed kernel oil increased with increase of pressure from 100 to 200 bar and decreased with increase of temperature from 40 to 60 °C.

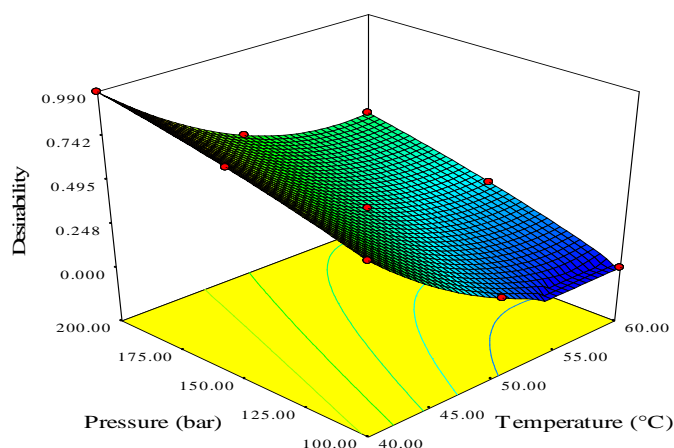


Figure 1: Effect Of SC-CO₂ Pressure and Temperature on Desirability of Extraction of Moringa (PKM-1) Seed Kernel Oil

Verification of Predicted Variables for SC-CO₂ Extracted Moringa (PKM-1) Seed Kernel Oil

The suitability of the model equation for predicting the optimum response values was verified using the optimal condition. The experimental sample had the optimum process conditions of temperature (40 °C) and pressure (200 bar). This optimized parameter was carried out thrice and the average values of each response are presented. Verification of the predicted and actual responses of moringa (PKM-1) seed kernel oil is presented in Table 2. From the table, it is seen that, the % relative deviation modulus of 0.29 and 0.35 for extraction yield and efficiency respectively were less than 0.5 with desirability of 0.98. This implies the process of extraction at optimum conditions is highly efficient.

Table 2: Verification of Predicted and Actual Process Parameters and Responses of SC-CO₂ Extraction of Moringa (PKM-1) Seed Kernel Oil

	Parameters	Predicted Value	Experimental Value	% Relative Deviation Modulus
	Temperature (°C)	40.00	40.00	-
	Pressure (bar)	200.00	200.00	-
	Extraction yield (g/100 g)	34.44	37.17	0.29
	Extraction efficiency (%)	88.85	97.10	0.34
Desirability at optimum process conditions : 0.98				

Cost of Production of Oil from Moringa Seed Kernel Powder Using SFE Equipment

Estimation of cost of production of moringa (PKM-1) seed kernel oil was obtained as Rs. 13,652 /- per kg of oil with benefit cost ratio of 1.83:1.

CONCLUSIONS

SC-CO₂ extracted *Moringa oleifera* (PKM-1) seed kernel oil yield and extraction efficiency decreased with increase in extraction temperature and increased with increase in pressure. The optimum condition for SC-CO₂ extraction of moringa (PKM-1) seed kernel oil was found to be SC-CO₂ pressure of 200 bar and temperature of 40 °C. The desirability of the process at optimum conditions was found to be 0.98. The production cost of oil from moringa (PKM-1) seed kernel powder using supercritical fluid extraction equipment was found to be Rs. 13,652 /- per kg of oil with benefit

cost ratio of 1.83:1. Hence, the developed technology could be adopted for commercial extraction of moringa (PKM-1) seed kernel oil.

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